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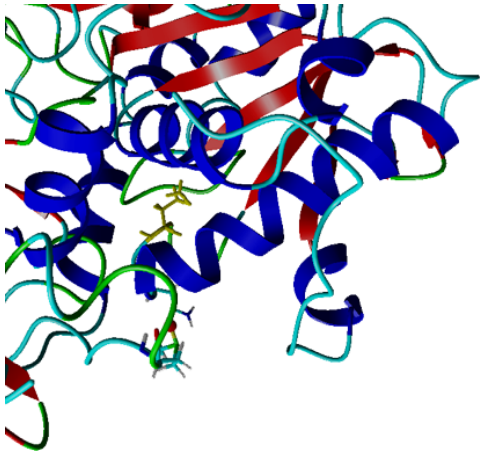
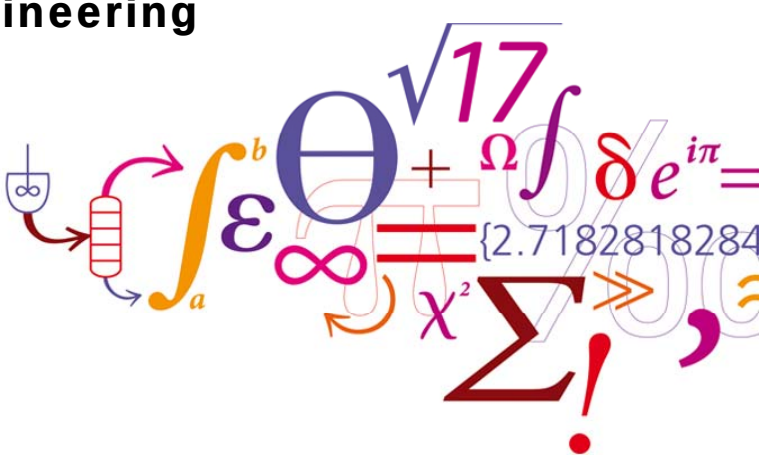


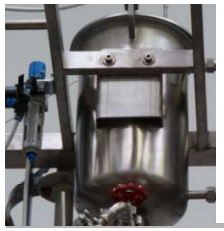
Enzyme stability: Process engineering requirements

Ulrika Törnvall, Mathias Nordblad, Pär Tufvesson and John M Woodley

Center for Process Engineering and Technology
Department of Chemical and Biochemical Engineering
Technical University of Denmark
Kgs Lyngby
Denmark



DTU Chemical Engineering
Department of Chemical and Biochemical Engineering



Outline

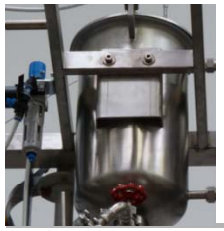
Introduction to the process perspective

Enzyme operational stability

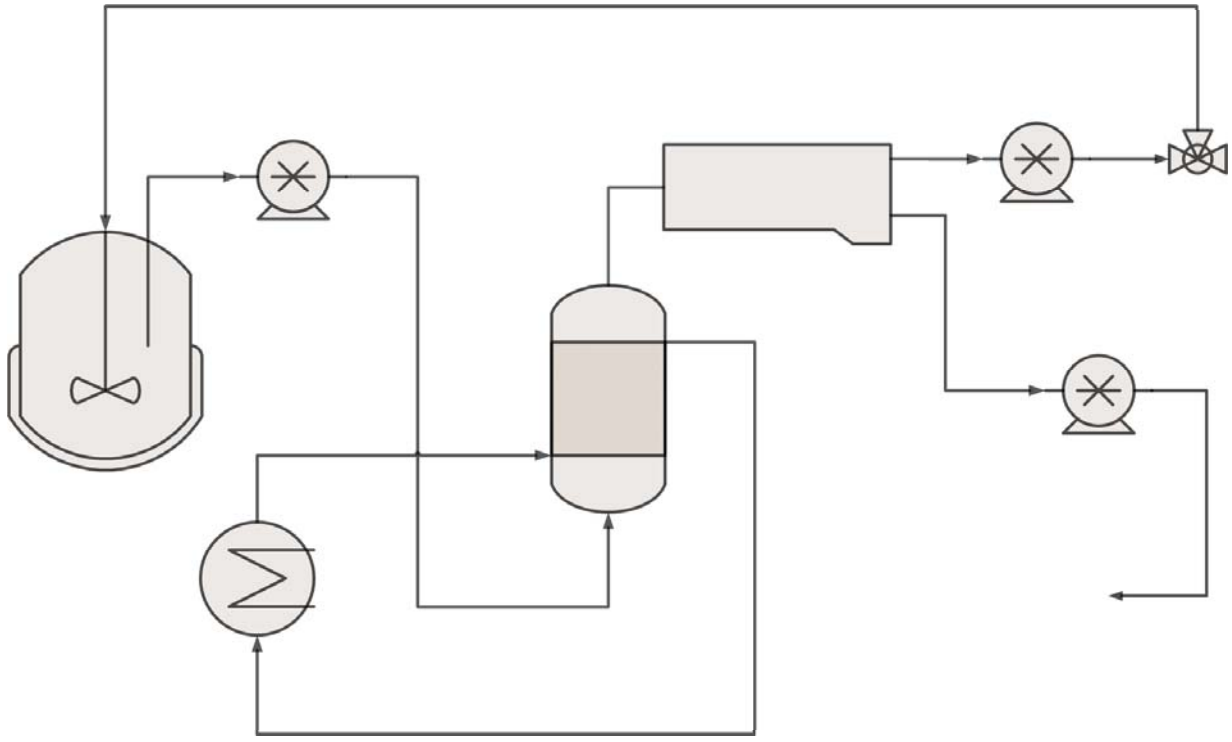
- Process development strategy
- Stabilization methods
- The search for the deactivation mechanism
- Measurement of operational stability

Case study: Chemo-enzymatic epoxidation

Take home messages



Demands on the enzyme



Thermal stability

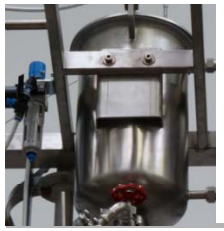
Chemical stability

- Solvents
- pH
- Substrates
- Products

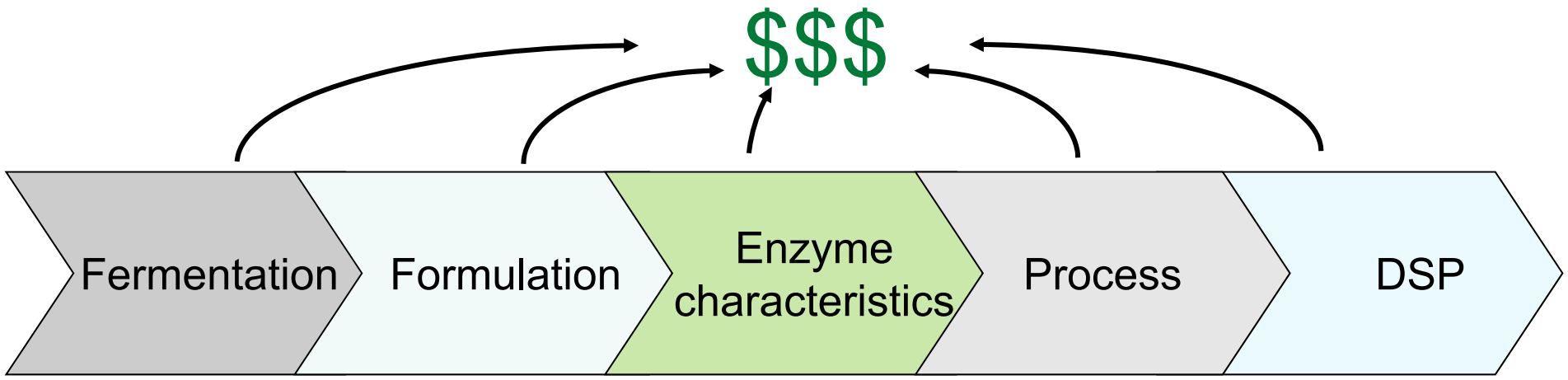
Mechanical stab.

- Stirring
- Pumping

Interfaces

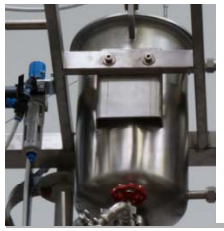


The cost-effective process

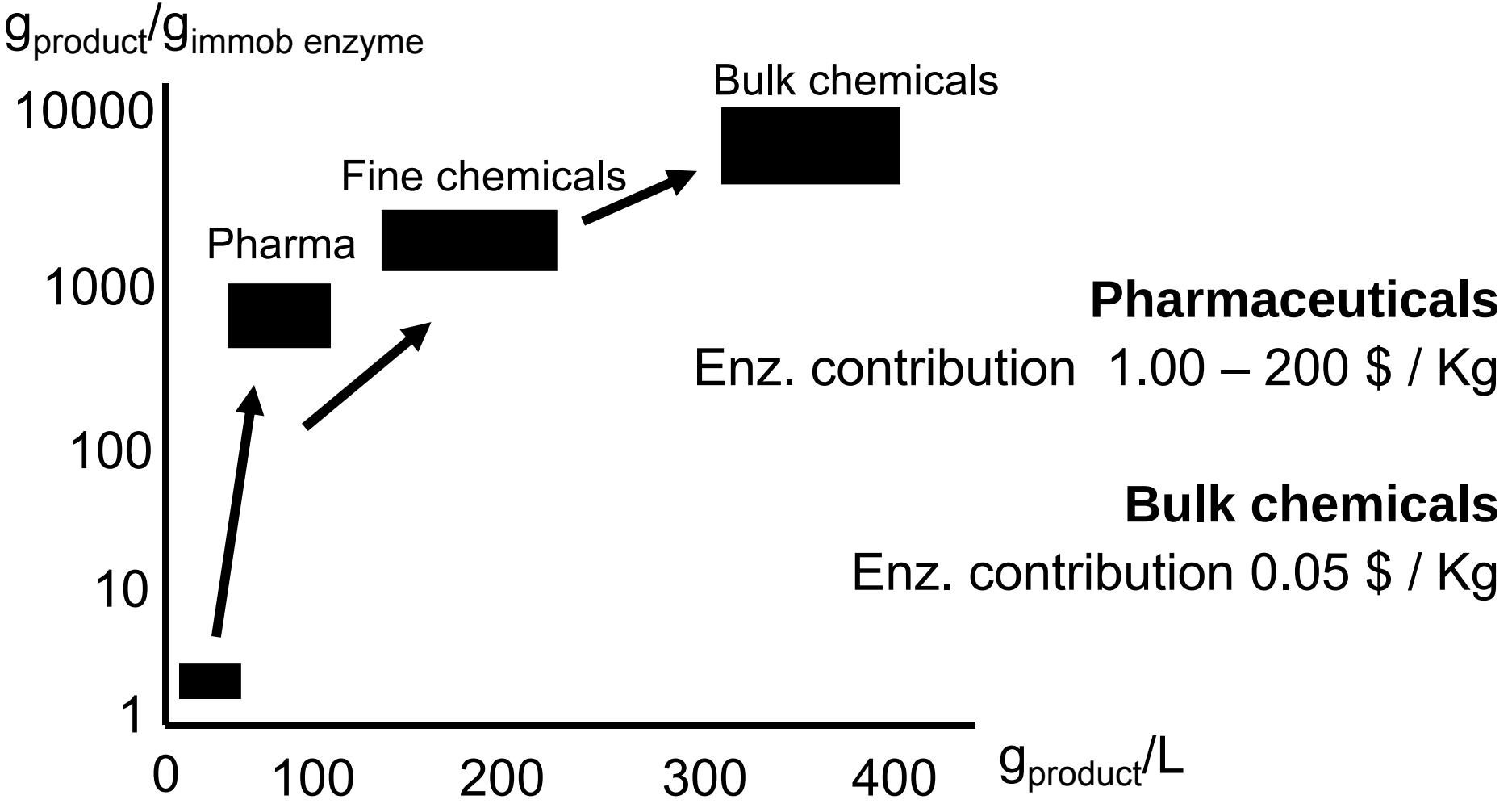


- Expression level (g/L) - Time - Scale	- Carrier and/or - Crosslinker - Procedure	- Activity - Selectivity - Stability	- Substrate - Energy - Equipment	- Chemicals - Energy - Equipment

See also Tufvesson *et al* (2011)
Org. Proc. Res Dev. **15** 266



Productivity targets



Pharmaceuticals

Enz. contribution 1.00 – 200 $ / Kg

Bulk chemicals

Enz. contribution 0.05 $ / Kg

Process development strategy

Economic constraints
Process constraints

→ Operational window (upper and lower limits for temperature, pH, etc)
Does biocatalysis seem feasible?

↓

Scrutinize market/literature for available enzymes (active and stable within operational window)

→ Best available candiate(-s)

↓

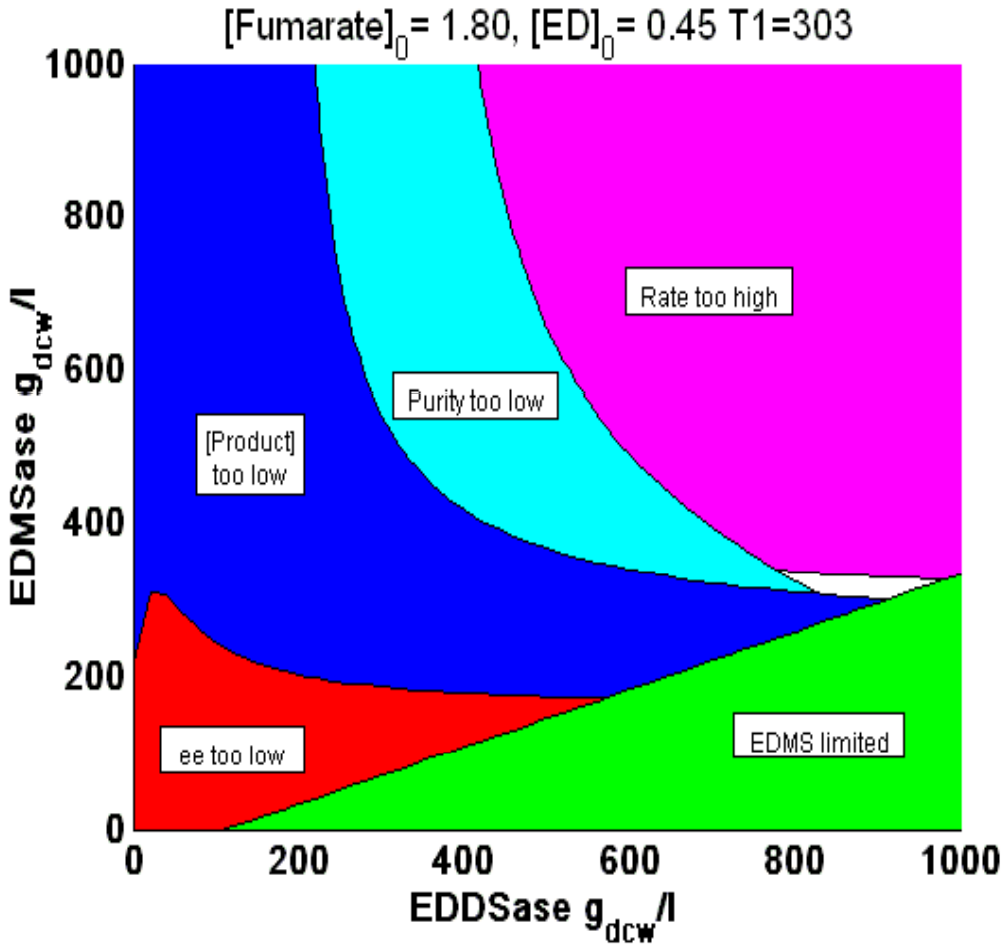
Experiments in small scale with reaction media engineering, based on theoretically determined conditions

→ Process parameters

Process parameters influencing productivity

Metric	Equation	Hurdle
Maximum rate	$\left(\frac{d[P]}{dt}\right)_{max}$	< 15 $g.l^{-1}.hr^{-1}$
Product concentration	$P = [P]_{final}$	≥ 100 $g.l^{-1}$
Yield	$Y_{\frac{P}{F}} = \frac{[P]_{final}}{[F]_0} \times \frac{116}{292} \times 100$	≥ 50%
Enantiomeric excess	$ee = \frac{[P]_{final}}{[P]_{final} + [RP]_{final}}$	≥ 99%
Enzymatic efficiency	$\eta = \frac{[P]_{final}}{[E_1]}$	≥ 10 $g.g_{dcw}^{-1}$
Purity	$X = \frac{[P]_{final}}{[P]_{final} + [RP]_{final} + [RM]_{final} + [SM]_{final}} \times 100$	≥ 95%

Window for enzyme concentration



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Experiments in small scale with reaction media engineering, based on theoretically determined conditions

→ Process parameters
Deactivation causes
Are extra costs such as immobilization possible?

→ Enzyme stabilization procedure started

Stabilization methods for operational stability

Method	Reported stabilization work (%)
Immobilization	34
Exploring extremophiles, environmental samples or similar	23
Chemical modification of enzyme or other stabilization during enzyme formulation	12
Reaction media engineering	11
Process conditions (temp, pH)	8
Protein engineering	7
Computer aided simulation/modeling	3
Process design (substrate supply, reactor type)	2

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Immobilization
- Carrier and/or
- Crosslinking
- Procedure

Protein engineering
- Site-directed
- Random

Enzyme production
-Host
-Etc.

Explore extremophiles
Search for a better enzyme candidate

Process development strategy

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Process constraints

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Experiments in small scale with reaction media engineering, based on theoretically determined conditions

→ Process parameters
Deactivation causes
Are extra costs such as immobilization possible?

→ Enzyme stabillization procedure started

↓

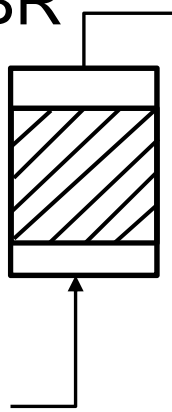
Process and economic model

→ Reactor configuration

Enzyme stability considerations during process design and scale-up

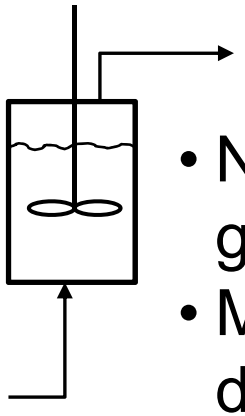
Reactor type

PBR



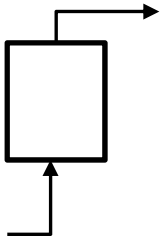
- Concentration gradient
- No mechanical disruption

CSTR

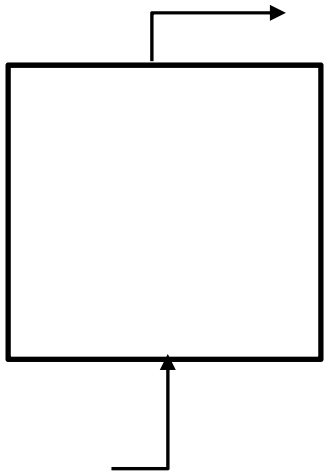


- No concentration gradient
- Mechanical disruption

Scale-up



- No concentration or temperature gradients



- Concentration and temperature gradients

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→ Process parameters
Deactivation causes
Are extra costs such as immobilization possible?

→ Enzyme stabilization procedure started

↓

Process and economic model

→ Reactor configuration

↓

Experiments according to model, in small/lab scale

→ Remaining deactivation causes
Optimal conditions for maximal productivity

The search for the deactivation mechanism

Pioneering work by Sadana, A. (1988) *Biotech. Adv.* **6** 340

First order, single step

$$E \xrightarrow{k_1} E_d$$

Series deactivation

$$E \xrightarrow{k_1} E_1 \xrightarrow{k_2} E_d$$

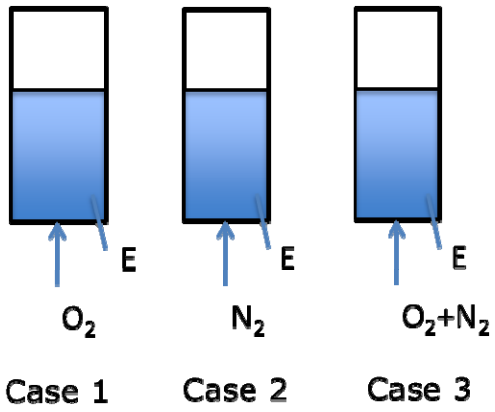
Reversible deactivation

$$E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_d$$

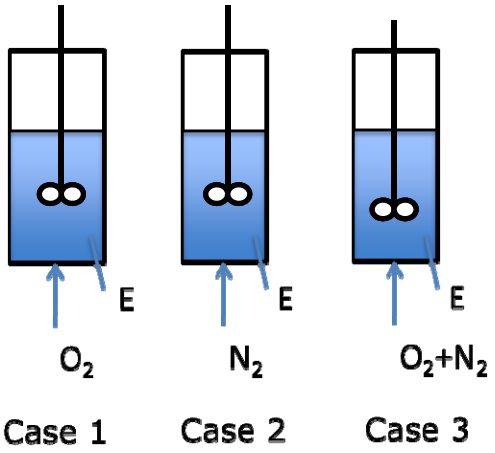
The search for the deactivation mechanism

Example: oxygen supply

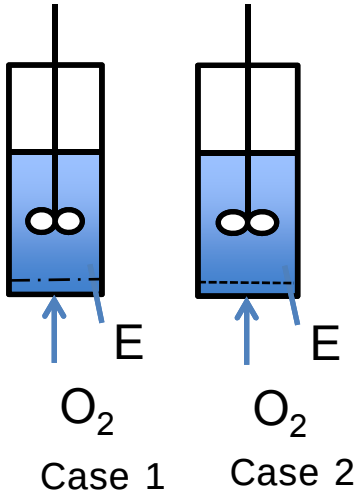
Gas supply



Mixing



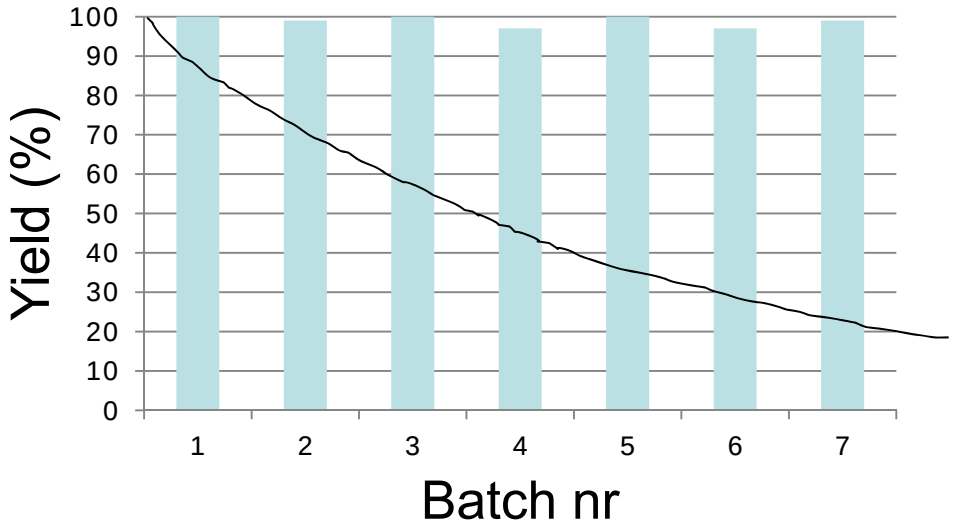
Bubble size



Measurement of operational stability

Half-life
Repeated batches

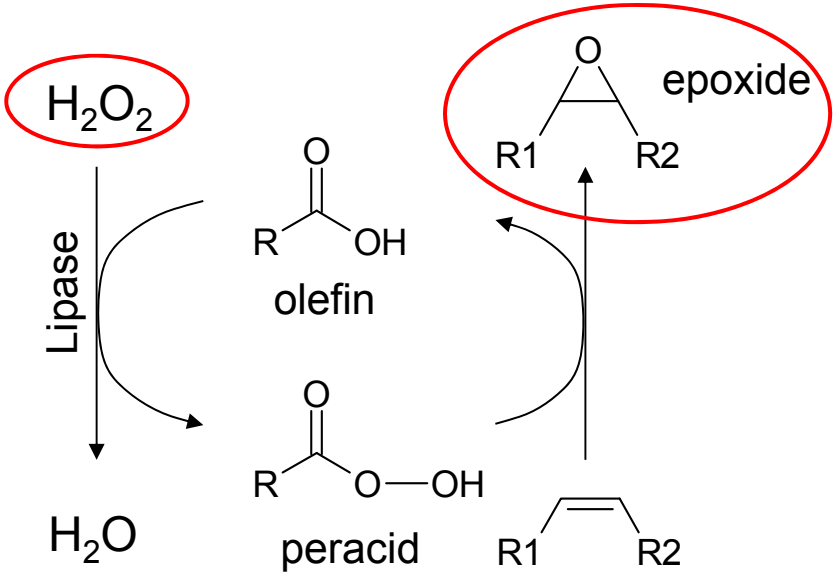
Apparent stability



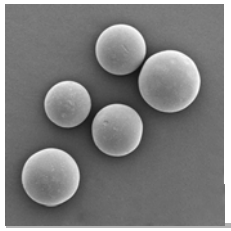
Suggestions
Process-like conditions
Report productivities
Continuously operated tank reactor
Fast procedures (e.g. T-ramping)

Stabilization work – case study

Chemo-enzymatic epoxidation



Törnvall *et al. (2007) Enzyme Microb. Tech.* **40** 447



Epoxidation - Stabilization efforts

- Type of enzyme
- Immobilization procedure
- Temperature
- H_2O_2 concentration and addition time
- Site-directed mutagenesis
- Fatty substrate
- Reactor design

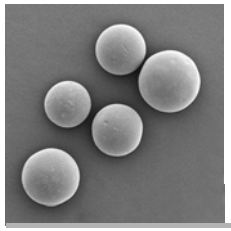
80-fold improvement still needed

Orellana-Coca *et al.* (2005) *Biocatal Biotransfor.* **23** 431

Törnvall *et al.* (2009) *Ind. Biotech.* **5** 119

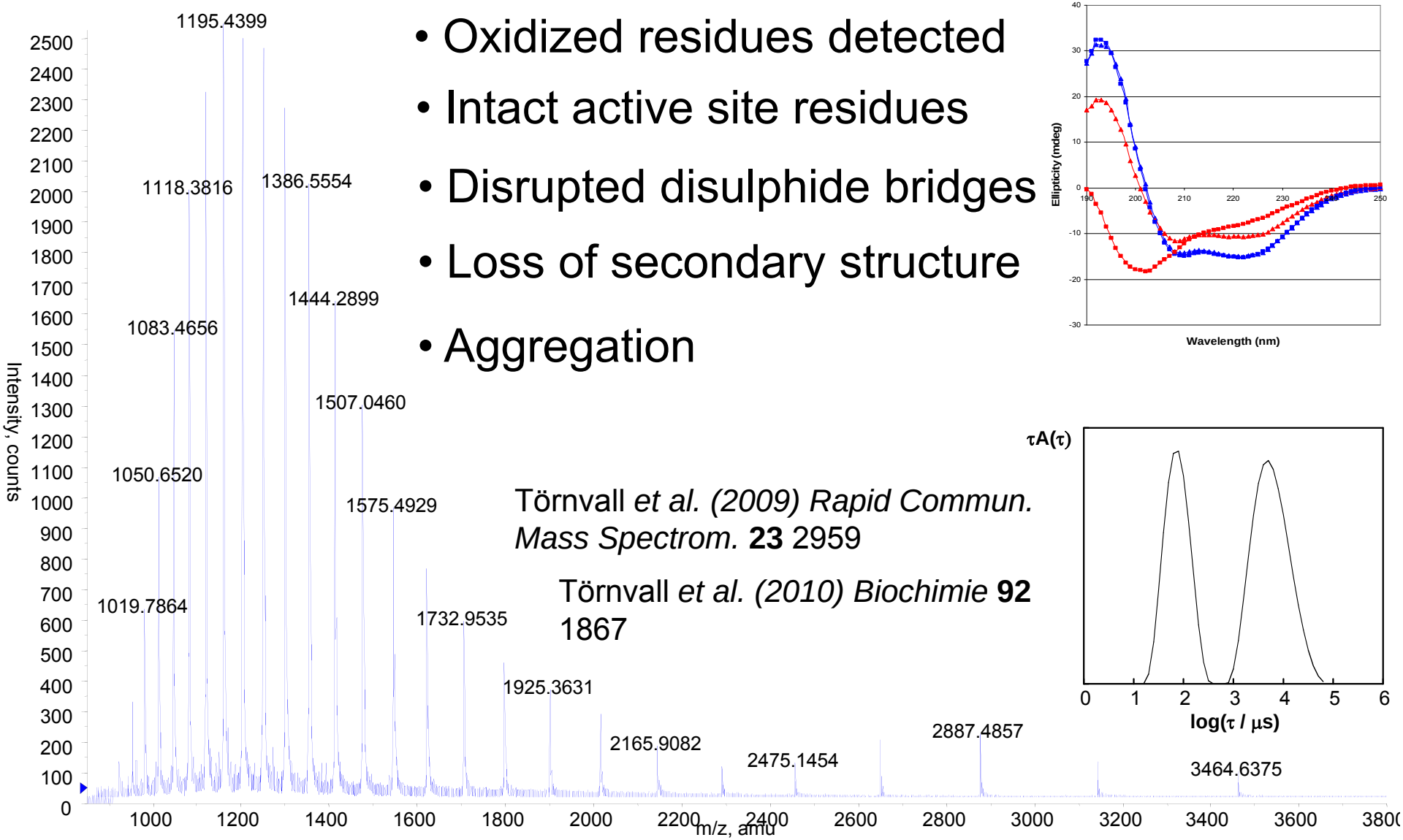
Hagström *et al. (2011) Biotechnol. Prog.* **27** 67

Törnvall *et al.* Unpublished data.



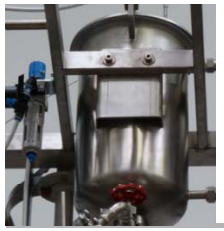
Deactivation mechanism

- Oxidized residues detected
- Intact active site residues
- Disrupted disulphide bridges
- Loss of secondary structure
- Aggregation



Törnvall *et al. (2009) Rapid Commun. Mass Spectrom.* **23** 2959

Törnvall *et al. (2010) Biochimie* **92** 1867

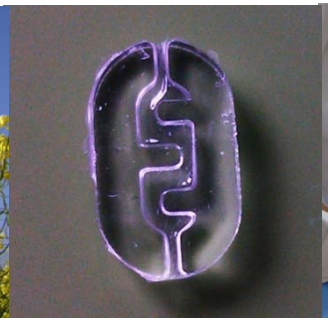


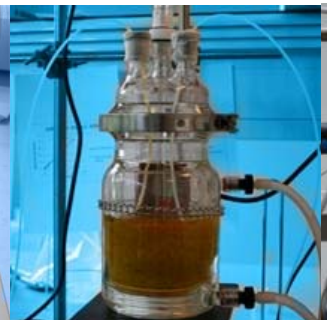
Take home messages

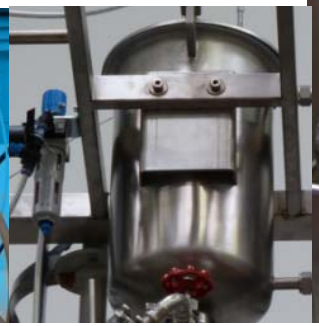
Report stabilization work as improved productivity, and include cost estimization

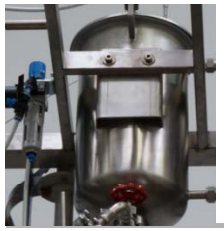
Screening procedures should always include the stability aspect

Avoid suboptimizations







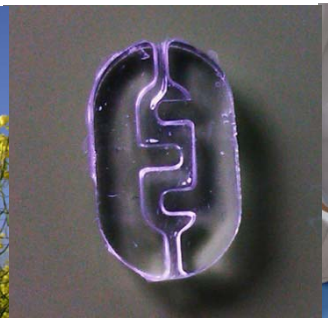


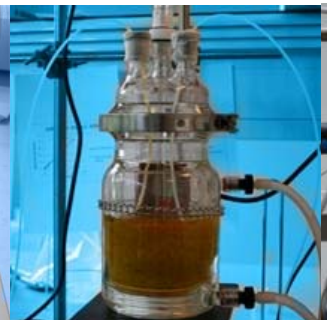
Take home messages

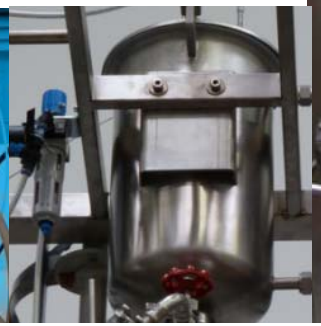
Process engineering is essential for implementation of bioprocesses

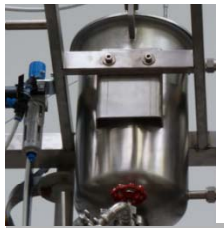
Process engineering tools are required to assist in designing the biocatalyst

We are always open to collaboration and student/faculty exchange









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Stuart Tindal

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Contact: ulrt@kt.dtu.dk